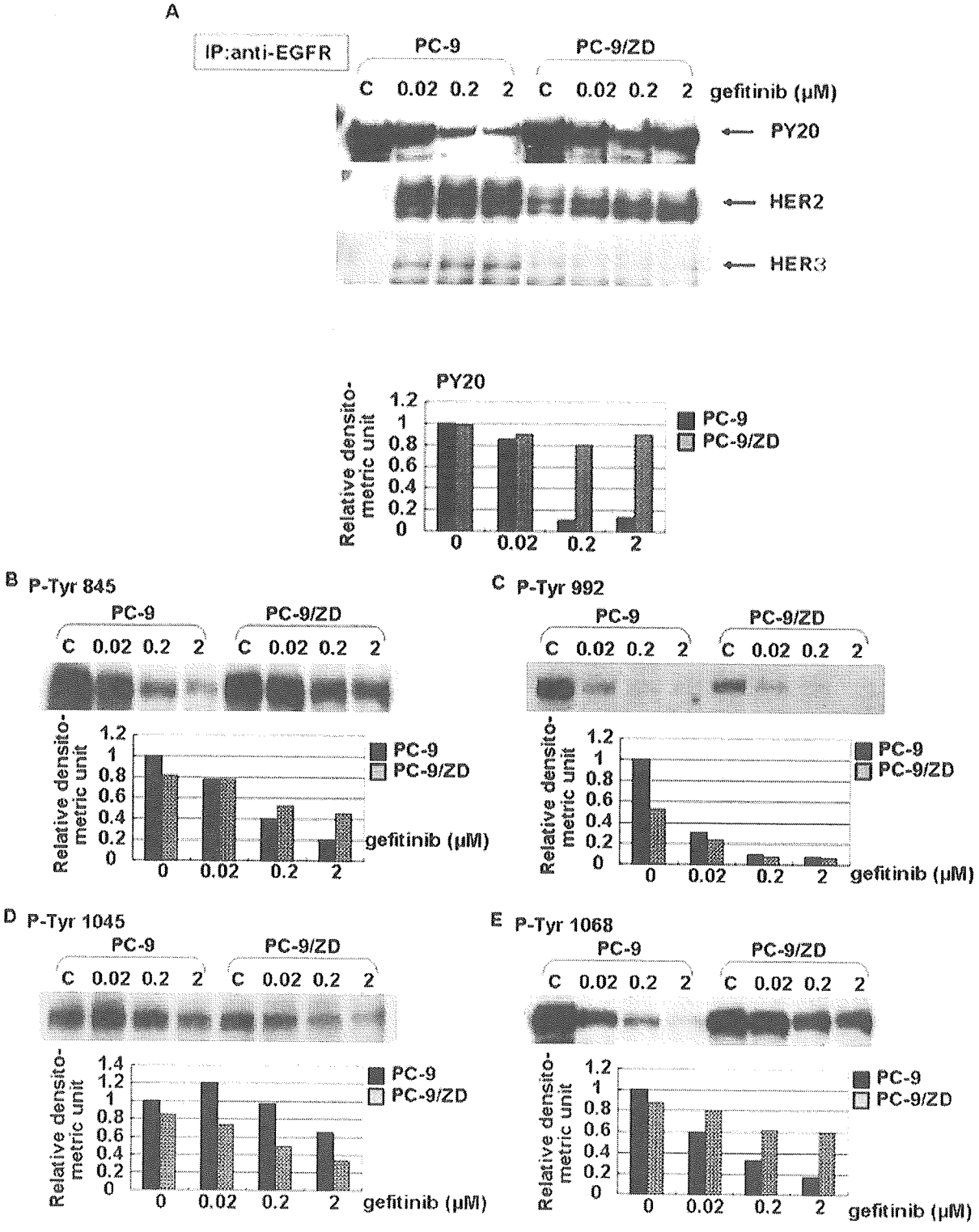

FIGURE 5 – Effect of gefitinib on autophosphorylation of EGFR. (*a*) PC-9 and PC-9/ZD cells (5×10^6) were exposed to 0.02, 0.2 or 2 μM gefitinib for 6 hr. The 1,500 μg of total cell lysate was immunoprecipitated with an anti-EGFR antibody. The immunoprecipitates were subjected to gel electrophoresis and Western blotting with anti-phosphotyrosine, anti-HER2 and anti-HER3 antibodies. Tyrosine-phosphorylated EGFR was determined with an anti-phosphotyrosine antibody. Heterodimer formation of EGFR was analyzed with anti-HER2 and anti-HER3 antibodies. The expression levels have been plotted in a graph. (*b–e*) PC-9 and PC-9/ZD cells were exposed to 0.02, 0.2 and 2 μM gefitinib for 6 hr. A 20 μg of protein of each sample was analyzed by Western blotting by using anti phospho-EGFR (Tyr845, Tyr992, Tyr 1045, Tyr 1068) antibodies.

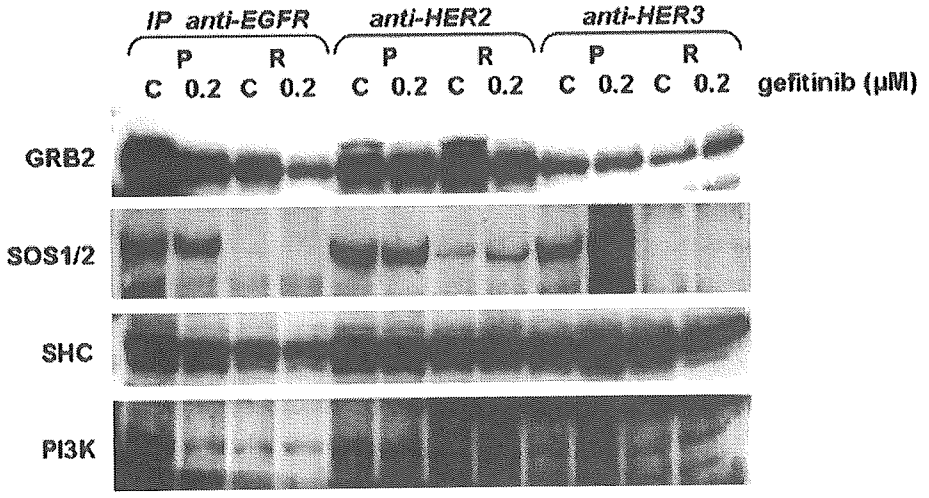

FIGURE 6 – Protein interaction between EGFR and its adaptor proteins. Cells (P: PC-9, R: PC-9/ZD) were exposed to 0 and 0.2 μM of gefitinib for 6 hr. The cells were lysed and immunoprecipitated with ant-EGFR, anti-HER2, and anti-HER3 antibodies, and the amounts of the Grb2, SOS1/2, SHC and PI3K precipitated were monitored by immunoblotting with their specific Abs.

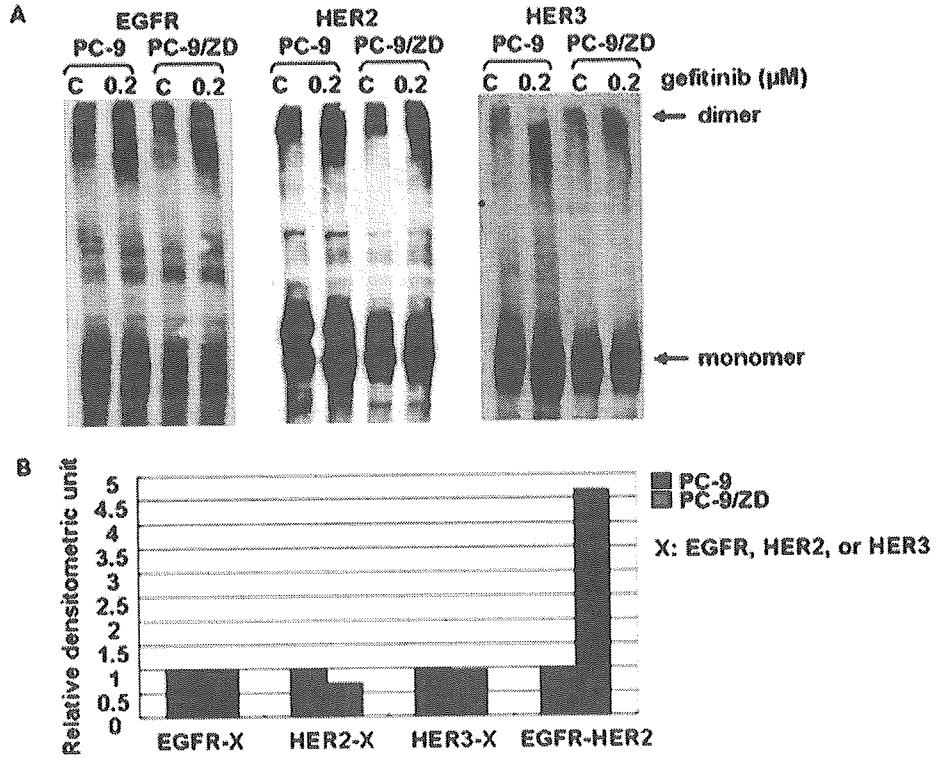

FIGURE 7 – Chemical cross-linking of PC-9 and PC-9/ZD cells. (*a*) After 6 hr exposure to 1.5 mM bis (sulfosuccinimidyl) substrate dissolved in PBS as indicated in Material and Methods. The cross-linking reaction was quenched and the cell lysates were prepared and subjected to immunoblot analysis of EGFR, HER2 and HER3. (*b*) Ratio of dimmers formed by PC-9 cells to those by PC-9/ZD cells in the absence of gefitinib. The density of the bands in (*a*) for EGFR-X, HER2-X and HER3-X were quantified densitometrically. The ratio of EGFR-HER2 was calculated by the band density obtained in Figure 5*a*. X = EGFR, HER2 or HER3.

Discussion

Interest in resistance to target-based therapy (TBT) has been growing ever since clinical efficacy was first demonstrated.[11–13] Although CML patients respond to STI-571 well at first, most patients eventually relapse in the late stage of the disease.[25–27] It has been reported that some patients in whom treatment with gefitinib is effective at first, ultimately become refractory.[30] Resistance is likely to remain a hurdle that limits the long-term effectiveness of TBT. PC-9 had a deletion mutation within the kinase domain of *EGFR* and is highly sensitive. These characters are similar to those of NSCLC with clinical responsiveness to gefitinib. Analyzing the mechanism of resistance of PC-9/ZD subline might be clinically meaningful.

The mechanism of drug resistance is thought to be multifactorial. Because the growth-inhibitory assay in our present study showed no cross resistance to a variety of cytotoxic agents, the mechanism of the resistance differs from the mechanism of multidrug resistance patterns. Although expression of BCRP, one of the multidrug-resistance-related proteins has been reported to contribute to the resistance to gefitinib,[31] expression of *BCRP* mRNA is observed only in PC-9 cells (data not shown). Although mutations in the ATP-binding pocket of *BCR-ABL* gene have been identified recently in cells from CML patients who were refractory to STI-571 treatment or relapse,[25–27] there have been no reports of any such mutations for gefitinib resistance. PC-9/ZD also became refractory to gefitinib without secondary mutation in *EGFR* cDNA. These suggest the possibility of refractory tumor after treatment of gefitinib including this kind of phenotype.

There is no significant difference in expression level of EGFR between PC-9 and PC-9/ZD. Does the antitumor effect of gefitinib

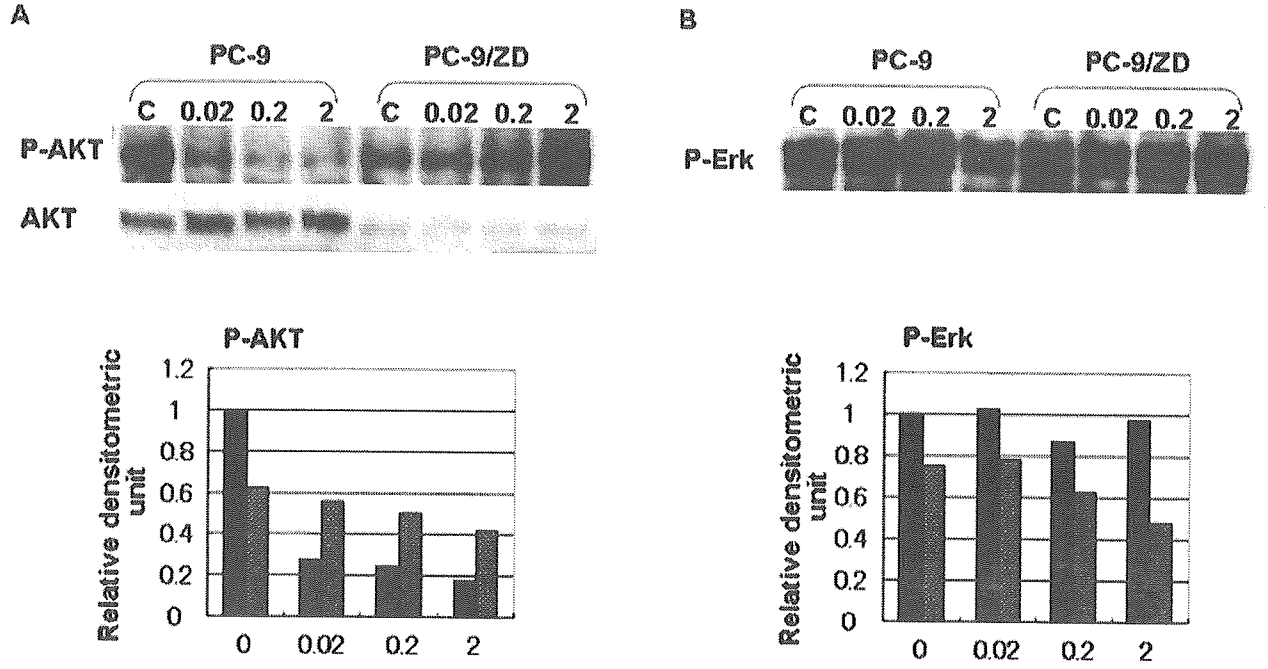

FIGURE 8 – Effect of gefitinib on the MAPK and AKT pathway. Cells were placed in medium containing 0, 0.02, 0.2 or 2 μM of gefitinib for 6 hr and harvested in EBC buffer. Total cellular lysates were separated on SDS-PAGE, transferred to a membrane and blotted with (*a*) anti-phospho-AKT (Ser473) and (*b*) anti-phospho-Erk (p44/42) antibodies. The expression levels are shown in a graph.

require EGFR expression? Naruse *et al.*[32] suggested that the high sensitivity of K562/TPA to gefitinib is due to acquired EGFR expression. In their study autophosphorylation of EGFR in K562/TPA cells was inhibited by 0.01 μM gefitinib, and the IC_{50}-value of gefitinib in parental K562 cells, which do not express EGFR, was approximately 400-fold higher than that in the K562/TPA subline. Furthermore, most patients who responded to gefitinib therapy have *EGFR* mutation in lung tumor.[18,19] These findings suggest strongly that gefitinib exerts its antitumor effect through an action on EGFR. Our present study showed similar EGFR expression and autophosphorylation levels in PC-9 and PC-9/ZD cells. The inhibitory effect of gefitinib on phosphorylation of EGFR is different. PC-9/ZD did not show cross-resistance to the specific EGFR TK inhibitors RG-14620 and Lavendustin A in an MTT assay, nor did inhibit the phosphorylation of EGFR at the cellular level (data not shown). Paez *et al.*[18] reported that phosphorylation of EGFR in gefitinib-resistant cell lines was inhibited only when gefitinib was present at high concentration. These findings suggest that the difference in the inhibitory-effect on EGFR phosphorylation may determine the efficacy of the drug.

The inhibitory effect of gefitinib on EGFR phosphorylation is not significant in PC-9/ZD cells despite the absence of differences in the sequences of *EGFR*, *HER2*, and *HER3*. There are several possible explanations for the difference in inhibitory effect. First, the avidity of gefitinib for the ATP-binding site of EGFR may be decreased in PC-9/ZD cells due to a protein-protein interaction, *i.e.*, EGFR and a certain protein prevent gefitinib from binding to EGFR. Second, a change in the activity of specific protein-tyrosine kinase or phosphatase of EGFR in PC-9/ZD cells, especially after exposure to gefitinib, may result in resistance to inhibition of EGFR phosphorylation. The phosphorylation level is maintained in exquisite balance by the reciprocal activities of kinase and phosphatase,[33,34] and Wu reported that phosphatase plays a role in STI571-resistance.[35] Third, increased heterodimer formation by EGFR with other members of the HER family results in the limited inhibition. Heterodimer formation is increased in PC-9/ZD cells under basal conditions, and no increase in formation was observed after exposure to gefitinib, although marked heterodimer induction was observed in PC-9 cells. Calculations in *in vitro* studies have shown that the IC_{50}-value for inhibition of the tyrosine kinase activity of EGFR is 0.023–0.079 μM, whereas the IC_{50}-value for inhibition of HER2 is 100-fold higher.[36] We estimate that the inhibitory effect of gefitinib depends on the ratio of homodimer formation to heterodimer formation, and the heterodimer may be one of the routes of escape from the action of gefitinib.

Signal transduction by the HER family member is mediated by 2 major pathways, the MAPK signaling pathway and the AKT signaling pathway, which regulate cell proliferation and survival. Because phosphorylated AKT was inhibited completely by gefitinib in PC-9 cells, but inhibition of phosphorylated MAPK was not significant, inhibition of the AKT pathway may be more important to cell sensitivity than inhibition of MAPK. Moasser *et al.*[37] reported consistent results, showing that downregulation of AKT activity is predominantly seen in tumors that are sensitive to gefitinib. The phosphorylation of AKT and MAPK was not inhibited significantly by gefitinib in PC-9/ZD cells. This finding might be attributable to inactivation of Tyr 1068-GRB2-SOS-mediated signaling.

Based on the results of this comparative study, EGFR-GRB2-SOS complex formation, phosphorylation of Tyr1068, the ratio of the amount of homodimer formation to heterodimer formation, and the AKT signaling pathway are possible predictive biomarkers for gefitinib sensitivity. As a different approach, we are now looking for the genes associated with gefitinib resistance in PC-9/ZD cells compared to PC-9 cells by subtractive cloning.

Acknowledgements

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Pilot Study of Concurrent Etoposide and Cisplatin Plus Accelerated Hyperfractionated Thoracic Radiotherapy Followed by Irinotecan and Cisplatin for Limited-Stage Small Cell Lung Cancer: Japan Clinical Oncology Group 9903

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Abstract

Purpose: Irinotecan and cisplatin (IP) significantly improved survival compared with etoposide and cisplatin (EP), in patients with extensive-stage small cell lung cancer (SCLC) in a previous Japan Clinical Oncology Group (JCOG) randomized trial. JCOG9903 was conducted to evaluate the safety of sequentially given IP following concurrent EP plus twice-daily thoracic irradiation (TRT) for the treatment of limited-stage SCLC (LSCLC).

Experimental Design: Between October 1999 and July 2000, 31 patients were accrued from 10 institutions. Thirty patients were assessable for toxicity, response, and survival. Treatment consisted of etoposide 100 mg/m^2 on days 1 to 3, cisplatin 80 mg/m^2 on day 1, and concurrent twice-daily TRT of 45 Gy beginning on day 2. The IP regimen started on day 29 and consisted of irinotecan 60 mg/m^2 on days 1, 8, and 15 and cisplatin 60 mg/m^2 on day 1, with three 28-day cycles.

Results: There were no treatment-related deaths. The response rate was 97% (complete response, 37%; partial response, 60%). Median overall survival was 20.2 months; 1-, 2-, and 3-year survival rates were 76%, 41%, and 38%, respectively. Of the 24 patients who started the IP regimen, 22 received two or more cycles. Hematologic toxicities of grade 3 or 4 included neutropenia (67%), anemia (50%), and thrombocytopenia (4%). Nonhematologic toxicities of grade 3 or 4 included diarrhea (8%), vomiting (8%), and febrile neutropenia (8%). Of the 20 patients with recurrence, none had local recurrence alone and only two had both local and distant metastasis as the initial sites of disease progression.

Conclusions: IP following concurrent EP plus twice-daily TRT is safe with acceptable toxicities. A randomized phase III trial comparing EP with IP following EP plus concurrent TRT for LSCLC is ongoing (JCOG0202).

Despite efforts to curb smoking, lung cancer remains the leading cause of cancer deaths in many industrialized countries. Small cell lung cancer (SCLC) accounts for about 15% of all lung cancer histology. Whereas combination chemotherapy is the cornerstone of SCLC treatment, meta-analyses showed that adding thoracic radiotherapy to combination chemotherapy significantly improves the survival of patients with limited-stage SCLC (LSCLC; i.e., disease confined to the hemithorax; refs. 1, 2). Several randomized trials have shown that early use of concurrent thoracic radiotherapy is superior to sequential or late use when etoposide and platinum are employed as combination chemotherapy (3–5). An intergroup phase III study showed accelerated hyperfractionated radiotherapy with etoposide and cisplatin (EP) to be superior to standard fractionation, with 5-year survival rates of 26% and 16%, respectively (6). Although substantial progress has been made during the past two decades, many LSCLC patients experience tumor recurrence and succumb to the disease, indicating the need for improved LSCLC therapy.

The Japan Clinical Oncology Group (JCOG) previously conducted a randomized phase III trial comparing irinotecan and cisplatin (IP) with EP in patients with extensive-stage SCLC. The response rate and overall median survival were significantly better for IP (i.e., 84.4% and 12.8 months with IP versus 67.5% and 9.4 months with EP, respectively). The 2-year

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survival rates were 19.5% for IP and 5.2% for EP (7). These encouraging results prompted us to explore the use of IP in LSCLC. We therefore undertook a pilot study to evaluate the safety of IP following concurrent EP plus twice-daily thoracic irradiation (TRT) for LSCLC.

Experimental design

Eligibility criteria. Patients with histologically or cytologically documented LSCLC, defined as disease confined to one hemithorax including bilateral supraclavicular nodes, were enrolled in this study. Additional eligibility criteria consisted of measurable or assessable disease, age <75 years, Eastern Cooperative Oncology Group performance status of 0 to 2, no previous treatment, leukocyte count $\geq 4{,}000/mm^3$, platelet count $\geq 10^5/mm^3$, hemoglobin ≥ 9.5 g/d, serum creatinine ≤ 1.5 mg/d, creatinine clearance ≥ 60 mL/min, serum bilirubin ≤ 1.5 mg/d, serum transaminase $\leq 2 \times$ ULN, and $PaO_2 \geq 70$ mm Hg. Exclusion criteria included active infection, uncontrolled heart disease or a history of myocardial infarction within the previous 3 months, interstitial pneumonia/active lung fibrosis on chest X-ray, peripheral neuropathy, malignant pleural or pericardial effusion, diarrhea, intestinal obstruction or paralysis, and active concomitant malignancy. The TRT portal should be no more than half of the hemithorax. No prior chemotherapy or radiotherapy was permitted. Pregnant or lactating women were excluded. Before enrollment in the study, each patient provided a complete medical history and underwent physical examination, blood cell count determinations, arterial blood gas, biochemical laboratory examinations, chest X-ray, electrocardiogram, chest computed tomographic scan, and whole-brain computed tomographic or magnetic resonance imaging, abdominal ultrasound and/or computed tomographic, and isotope bone scans. Blood cell counts, differential white counts, and other laboratory data were obtained weekly during each course of chemotherapy. All patients were reassessed at the end of treatment in the same manner as at the time of enrollment.

Treatment plan. Induction chemotherapy consisted of cisplatin 80 mg/m^2 on day 1 and etoposide 100 mg/m^2 on days 1 to 3. TRT was begun on day 2 of the induction chemotherapy and given twice daily (1.5 Gy per fraction, with ≥ 6 hours between fractions) and directed to the primary tumor for a total dose of 45 Gy in 3 weeks. The initial field included the primary disease site with a 1.5-cm margin around the mass, the ipsilateral hilum, the entire width of the mediastinum, and the supraclavicular lymph nodes (only if there was nodal tumor involvement). TRT was done with linear accelerators and the energy was 6 to 10 MV photons. After the administration of 30 to 36 Gy, the radiation field was reduced around the primary tumor and involved lymph nodes using parallel opposed oblique fields to limit the dose to the spinal cord and protect the uninvolved lung field. Following chemoradiotherapy, patients were treated with three cycles of IP. The IP regimen started on day 29 and consisted of irinotecan 60 mg/m^2 on days 1, 8, and 15 and cisplatin 60 mg/m^2 on day 1, with three 28-day cycles. If the leukocyte count decreased to $<3{,}000/mm^3$ or the platelet count fell below $100{,}000/mm^3$ on the first day of IP, chemotherapy was withhold until the counts recovered to $\geq 3{,}000/mm^3$ and $\geq 100{,}000/mm^3$, respectively. Administration of irinotecan was skipped on day 8 and/or 15 if the leukocyte count was $\leq 2{,}000/mm^3$, the platelet count was $\leq 50{,}000/mm^3$, or there was any diarrhea regardless of grade, or a fever of $\geq 37.5°C$. The dose of irinotecan in subsequent cycles was reduced by 10 mg/m^2 from the planned dose if grade 4 hematologic toxic effects or grade 2 or 3 diarrhea developed. Administration of granulocyte colony-stimulating factor was prohibited on the days of chemotherapy or radiotherapy. Primary prophylactic granulocyte colony-stimulating factor was not given. For patients who had developed grade 4 neutropenia during the previous cycles of chemotherapy, secondary prophylactic granulocyte colony-stimulating factor administration was allowed. Prophylactic antibiotics were not given.

Treatment was discontinued in patients with grade 4 nonhematologic toxicity. Prophylactic cranial irradiation (25 Gy in 10 fractions) was conducted for patients showing a complete response or near complete response defined as a reduction of >90% in the sum of the products of the greatest perpendicular dimensions of bidimensional lesions. Tumor responses were assessed radiographically. Standard WHO response criteria (8) were used, and all responses were confirmed ≥ 28 days after initial documentation of the response. JCOG criteria were used to assess toxicity (9). JCOG criteria are similar to those of the National Cancer Institute Common Toxicity Criteria (10). Esophageal toxicity was graded as follows: grade 3, moderate to severe ulceration and edema, cannot eat, requires narcotic drugs; grade 4, serious ulceration and edema, resulting in complete obstruction or perforation.

Statistical consideration. The primary objective of this study was to evaluate the safety and feasibility of sequential administration of IP following EP plus concurrent twice-daily TRT. Simon's optimal two-stage design was used to determine the sample size and decision criteria (11). The regimen would be considered feasible if two cycles or more of IP were completed without grade 4 nonhematologic toxicity or treatment related death in at least 90% of patients and not feasible if the completion rate was ≤70%. The required number of patients was estimated to be 27, with $\alpha = 0.05$ and $\beta = 0.80$. We determined the planned sample size for the study to be 30 patients accrued over 12 months, with 36 months of additional follow-up.

Time-to-progression was calculated from the date of entry into study until the date of documented progression or death (in the absence of progression). Survival was calculated from the protocol treatment start date until the date of death. Both intervals were determined by the Kaplan-Meier method.

The protocol was approved by the Clinical Trial Review Committee of JCOG and the Institutional Review Board of the participating institutions. All patients provided written informed consent.

Results

Patient characteristics. Between October 1999 and July 2000, 31 patients were accrued from 10 institutions. Patient characteristics are detailed in Table 1. Although eligible, no patients with a performance status of 2 were actually enrolled in this trial. Thirty-one patients ultimately participated. One patient did not receive the protocol treatment because of a problem with the radiation equipment in the institution providing treatment. Thus, this patient was not evaluable.

Adherence to treatment plan. All patients completed concurrent chemoradiotherapy. Six patients did not receive the IP regimen, because of disease progression, septic shock

Table 1. Patient characteristics

Patient registered	31
Assessable	30
Not assessable (not treated)	1
Median age (range)	64 (43-74)
Gender	
Male	27
Female	4
Performance status 0/1	8/23

during chemoradiotherapy, renal dysfunction, or leukocytopenia, and two refused IP. Of the 24 patients given the IP regimen, 22 received two cycles or more of IP. The reasons for terminating IP before the second treatment cycle were grade 4 diarrhea in one patient and refusal, not significant toxicity, in one patient. Of the 22 patients who received two cycles or more of IP, nine received the original planned dose. In five patients, dose reductions in the second cycle of IP were necessary, 11 patients skipped day 8 and/or 15 irinotecan, and one patient had a minor protocol violation. Fifteen patients required that the second cycle of IP be delayed for 1 to 14 days. Of 17 patients (58%) who received the entire treatment, the median time delay from the planned protocol was 4 days (range, 0-21 days). Six patients were able to start the third cycle of IP without delay, relative to the first cycle of IP.

Toxicity. Toxicities associated with concurrent chemoradiotherapy are summarized in Table 2. The major toxicity was neutropenia. One patient had febrile neutropenia and septic shock. The same patient experienced grade 3 fatigue and anterior chest pain. IP was well tolerated (Table 3), despite diarrhea, vomiting, and hematologic toxicities. One patient, who had grade 2 nausea/vomiting, refused further treatment after the first cycle of IP. Another patient, who refused days 8 and 15 irinotecan during the second cycle, had grade 2 diarrhea and nausea/vomiting. No grade 3 or 4 pulmonary toxicity was observed. There were no treatment-related deaths.

Table 2. Major toxicities concurrent EP/TRT ($n = 30$)

Toxicity	Grade 3, no. patients (%)	Grade 4, no. patients (%)
Hematologic		
Anemia	0	0
Leucopenia	13 (43)	15 (50)
Neutropenia	9 (30)	19 (63)
Thrombocytopenia	2 (7)	1 (3)
Nonhematologic		
Esophagitis	2 (7)	0
Infection	1 (3)	0
Hypotension*	0	1 (3)
Fatigue*	1 (3)	0
Anterior chest pain*	1 (3)	0
Febrile neutropenia	2 (7)	

*These events occurred in the same patient.

Table 3. Major toxicities irinotecan and cisplatin (IP) ($n = 24$)

Toxicity	Grade 2, no. patients (%)	Grade 3, no. patients (%)	Grade 4, no. patients (%)
Hematologic			
Anemia	6 (25)	12 (50)	0
Leucopenia	6 (25)	12 (50)	5 (21)
Neutropenia	5 (21)	12 (50)	5 (21)
Thrombocytopenia	5 (21)	1 (4)	0
Nonhematologic			
Diarrhea	4 (17)	1 (4)	1 (4)
Vomiting	3 (13)	2 (8)	0
Febrile neutropenia	—	2 (8)	0
Fever	2 (8)	0	0
Infection	4 (17)	0	0

Neither grade 2, or more severe, late radiation toxicities n radiation recall reactions were reported.

Response and survival. The overall response rate was 97 (complete response, 37%; partial response, 60%). Overall an progression-free survivals are depicted in Figs. 1 and 2 Th median follow-up time of all patients was 20 months and th for surviving patients 40 months. The median progression-fr survival was 9 months, and the median overall survival wa 20 months. The 24- and 36-month overall survivals were 41 and 38%, the 24- and 36- month progression-free survival 30% and 26%, respectively.

Pattern of relapse. First sites of disease progression ar presented in Table 4. Of the 18 patients who have died t date, all died of progressive disease. Surprisingly, no patien showed relapse solely at the local-regional site (within TR field). Only two patients had both local and distan involvement. There were 11 patients whose initial site o relapse was the brain. Of these, six had relapses solely in th brain. Whereas two patients had complete response an received prophylactic cranial irradiation, four had partia remission and did not receive prophylactic cranial irradiation

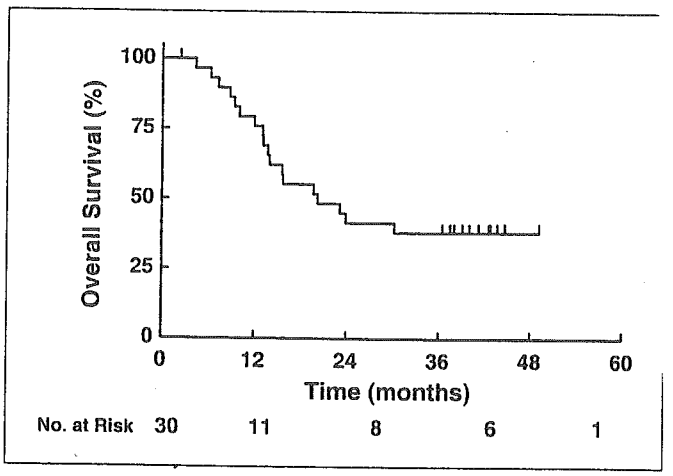

Fig. 1. Overall survival.

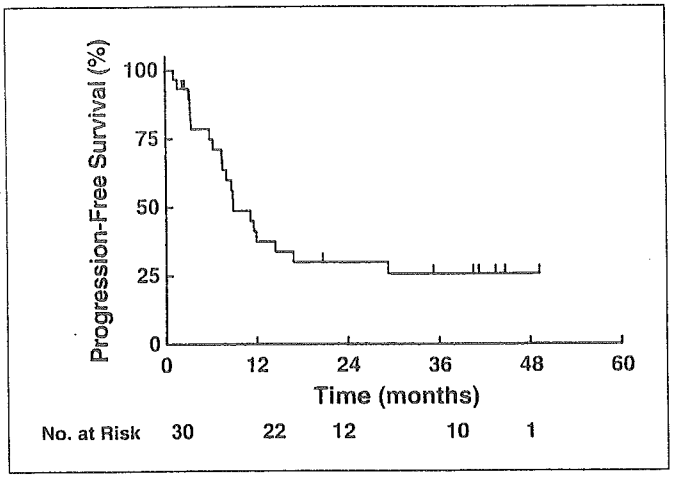

Fig. 2. Progression-free survival.

Other relapse sites included the liver in four patients, bone in three, pleural effusion in three, and supraclavicular lymph nodes in two.

Discussion

Irinotecan is one of the most active agents against SCLC (12). A phase II study of irinotecan and cisplatin yielded a response rate of 86% and median survival of 13.2 months in patients with extensive SCLC (13). A phase III study confirmed excellent results and showed IP to be more effective than etoposide and cisplatin in extensive SCLC (7). Three confirmatory trials, comparing IP with EP for extensive SCLC are ongoing in Europe and the United States. Although dose-finding studies to explore integrating irinotecan into the early concurrent phase of chemoradiation for LSCLC are also currently being conducted by the Radiation Therapy Oncology Group and other U.S. groups. The dose-finding JCOG study of concurrent use of IP with TRT in stage III non-small cell lung cancer showed that the full dose of irinotecan could not be given due to neutropenia, diarrhea, and pulmonary toxicity (14). Thus, we employed IP as a sequential treatment following EP plus concurrent TRT.

The present trial showed IP following concurrent EP plus twice-daily TRT to be safe, with acceptable toxicities. Hematologic toxicities and diarrhea, while on the IP regimen following concurrent chemoradiotherapy, are similar to those of a previous phase III trial conducted by JCOG (JCOG9503; ref. 7). Neither grade 3 or 4 pulmonary toxicity nor treatment related deaths were observed. The West Japan Thoracic Oncology Group conducted a similar phase II study of EP plus twice-daily TRT followed by IP for LSCLC (15). Promising response (88%) and 2-year survival (51%) rates were reported, with acceptable toxicities.

Table 4. Sites of first failure (n = 20)

Site	No. patient (%)
Isolated local-regional failure	0 (0)
Local-regional and distant	2 (10)
Distant	18 (90)
Brain only	6 (30)
Other sites of failure*	12 (60)

*Recurrence at sites other than the primary tumor or brain only.

Local failure is an important problem in the treatment of LSCLC. Turrisi et al. showed the rate of local failure to be reduced in the twice-daily TRT plus EP group as compared with the once-daily TRT plus EP group: the rate was 52% in the group receiving once-daily therapy and 36% in that receiving twice-daily therapy (6). Eighteen percent of patients who received EP plus concurrent twice-daily TRT had first progression within the thorax in the previous JCOG phase III trial (5). It is noteworthy that no patient relapsed solely at the local-regional site and only two patients had both local and distant involvement in the present trial. There may be an interaction between TRT and IP even given sequentially. Another possibility relates to recent improvements in radiotherapeutic techniques with better imaging of the target volume by chest computed tomographic. This possibility should be assessed in a future randomized trial.

It is important to integrate new active anticancer agents to the combined modality treatments for LSCLC. Irinotecan has been clearly shown to have clinical activity in a randomized trial, against extensive-stage SCLC. Several other new agents including targeted therapies have failed to show clinical activity against SCLC. Based on these considerations, we conducted a randomized phase III trial comparing EP with IP following EP plus concurrent TRT for the treatment of LSCLC (JCOG0202). In the JCOG0202, eligible patients were randomized after the completion of induction chemoradiotherapy. Although feasibility may be a limitation of the present study, improvements are anticipated with appropriate use of granulocyte colony-stimulating factor, antibiotics, and patient education.

In summary, irinotecan and cisplatin following EP plus concurrent twice-daily TRT is a safe and active regimen for LSCLC. The observed low rate of local recurrence is encouraging. A randomized phase III trial comparing EP with IP following EP plus concurrent TRT for the treatment of LSCLC is currently under way.

Acknowledgments

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Unusual Late Pulmonary Complication in a Child After Umbilical Cord Blood Transplantation

High-Resolution CT—Pathologic Correlation

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Abstract: We encountered a late pulmonary complication after umbilical cord blood transplantation (UCBT) that has not been previously reported. High-resolution CT (HRCT) findings of this disease were compared with the pathology. HRCT obtained on inspiration showed dilated thick-walled bronchioli, and innumerable centrilobular linear and branching structures in the bilateral middle and lower lobes. Neither mosaic perfusion nor air-trapping was seen in HRCT on inspiration and expiration. These HRCT findings were atypical compared with those of former bronchiolitis obliterans (BO) after bone marrow transplant (BMT). Pathologic specimens obtained by open lung biopsy showed thickening of the wall from the distal bronchioli to the alveolar ducts due to submucosal and intraepithelial infiltration of lymphocytes, histiocytes and foamy macrophages, which was not accompanied by organizing changes. These changes resemble lymphocytic bronchiolitis in lung transplant recipients, which was well correlated with HRCT findings. We think that our case was a new late pulmonary complication after UCBT.

Key Words: high-resolution CT, umbilical cord blood transplantation, lung complication, bronchiolitis, chronic graft-versus-host disease

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A wide variety of pulmonary complications occur in BMT recipients and are a major cause of morbidity and death.[1] Bronchiolitis obliterans and bronchiolitis obliterans with organizing pneumonia (BOOP) are known as fatal chronic graft-versus-host diseases (GVHD) occurring more than 100 days after BMT. Umbilical cord blood transplantation (UCBT) has been recently introduced as an alternative procedure instead of conventional BMT for patients with leukemia or lymphoma because HLA-matched bone marrow donors are insufficient.[2–4] High- resolution computed tomography (HRCT) is excellent in the detection of pulmonary abnormalities.[5] Several cases have been reported of obstructive lung disease after BMT evaluated by HRCT.[6,7] However, we could not find any reports of the HRCT appearance of lung disease after UCBT. Herein, we describe a case of late pulmonary complication after allogeneic UCBT in a child that focuses on the HRCT appearance and associated pathologic findings.

CASE REPORT

A 16-year-old girl was admitted to the children's hospital in July 2001 to receive induction full-dose chemotherapy for acute myelogenous leukemia (AML, M6), but did not achieve complete remission. In October 2001, after the preparative treatment consisting of total body irradiation, thiotepa, and cyclophosphamide (CsA), she received an umbilical cord blood unit from a male donor, and they were serologically one-antigen mismatched. CsA and short-term methotrexate were administered for the prevention of acute GVHD. Although the patient had evidence of grade II acute GVHD of the skin, hemorrhagic cystitis, and cytomegalovirus infection, she was discharged in May 2002.

When the patient was admitted to our hospital to receive treatment of chronic GVHD, cyclosporin A and low-dose predonisolone (PSL) were administered. However, she had signs and symptoms of patchy skin depigmentation, dryness of the eyes, anorexia, and diarrhea. She had a slight cough but no dyspnea. Since her symptoms had been deteriorating, she was admitted to the division of pediatrics of our hospital to examine whether the chronic GVHD had worsened.

On physical examination, the patient was pale and edematous. She had no fever, normal breathing, and a respiratory rate of approximately 12 breaths per minute. Breath sounds were normal on chest auscultation. A lung function test showed a pattern of restrictive lung disease and no obstructive pattern; the forced vital capacity (FVC) was 1.67 L, the forced expiratory volume in one second (FEV1.0) was 1.39 L, and FEV1.0/FVC was 83.2%.

Chest radiography on admission (Fig. 1) showed a bilateral hyperinflation and reticulonodular opacities. HRCT on inspiration showed dilated thick-walled bronchioli, and innumerable centrilobular linear and branching structures in the right middle and bilateral lower lobes (Fig. 2A,B). However, mosaic perfusion or air-trapping were not seen in HRCT on inspiration and expiration (Fig. 3A,B). We speculated that these HRCT findings were atypical compared with those of BO after BMT, and diffuse panbronchiolitis (DPB) was also considered in the differential diagnosis.

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This case was presented at the 2004 annual meeting of the Japanese Society of Thoracic Radiology.

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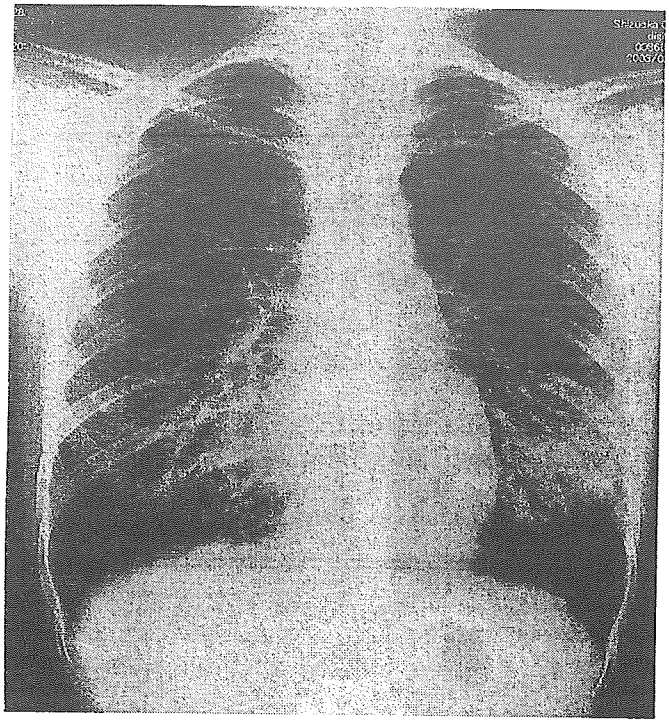

FIGURE 1. Admission chest radiograph shows bilateral hyperinflation and reticulonodular opacities that are predominantly hilar in distribution.

The patient received central intravenous nutrition and was administered an increased dose of CsA. After receiving the treatment, general conditions and laboratory data gradually improved. With the recovery of the gastrointestinal symptoms, her cough was decreasing but the chest radiographic findings were unchanged. Her clinical course, lung function test, and radiologic imaging were not compatible with BO after BMT. To determine the nature of her lung disease, open biopsy of the right middle lobe (segment 4b) and lower lobe (segment 8b) using video-associated thoracoscopy was performed with informed consent of the patient and parents. The laboratory data at the operation are shown in Table 1.

Pathologic specimens showed thickening of the wall of distal bronchioli due to submucosal and intraepithelial infiltration of lymphocytes and plasma cells (Fig. 4A,B). Foamy macrophages were also observed in the alveoli near thickened bronchioli. No organizing changes such as intraluminal fibrosis were seen. These findings were more conspicuous in segment 4b than in segment 8b. There was no evidence of bacterial or fungal infection. Therefore, the final pathologic diagnosis was bronchiolitis without organizing changes resembling lymphocytic bronchiolitis. After the administration of an increased dose of PSL, the cough and the HRCT findings were apparently improved (Fig. 5A,B).

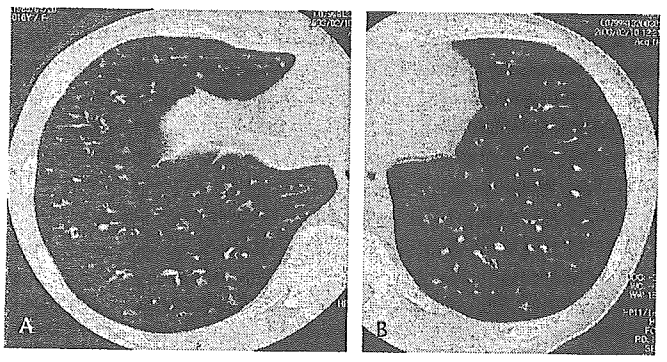

FIGURE 2. HRCT scan (1 mm collimation with high-resolution algorithm) on inspiration shows dilated thick-walled bronchioli, and innumerable centrilobular linear and branching structures in the right middle and bilateral lower lobes.

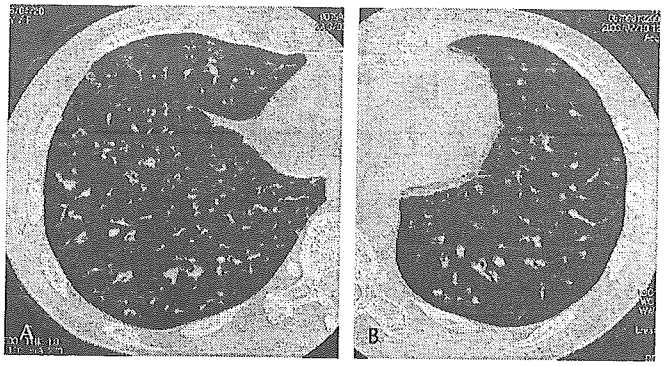

FIGURE 3. Mosaic perfusion or air-trapping are not seen in HRCT scan on expiration.

DISCUSSION

Chronic GVHD resembles an autoimmune disorder occurring 100 days after allogeneic transplantation and occurs in approximately 60–80% of long-term survivors of allogeneic hematopoietic cell transplant.[1] This immunologic complication is a major cause of morbidity and mortality, accounting for about one-quarter of the deaths in long-term survivors of transplants. Clinical manifestations of chronic GVHD are similar to autoimmune collagen vascular diseases, such as oral ulcerations, keratoconjunctivitis sicca, xerostomia, intrahepatic obstructive liver disease, and obstructive pulmonary disease. Our patient had many of these clinical manifestations.

Obstructive pulmonary disease after BMT was first reported in 1982.[8] BO occurs in up to 10% of BMT recipients but rarely after autologous transplantation. The clinical cause is uncertain but it considered the same as that of chronic GVHD. Some pathogenic mechanisms of fatal obstructive lung disease have been reported. Yousem proposed that GVHD insults to the lung might lead to a pattern of pulmonary scarring that is localized in the airways and perivascular zones.[9] On the other hand, Muller et al described that the primary pathologic process is an activated host immune response to the presence of viral antigen in the lung.[10] Usually, symptoms such as cough appear at 3 to 20 months after BMT and then progress to dyspnea, progressive airflow obstruction, and finally to respiratory failure. They respond poorly to corticosteroids and other immunosuppressive therapy and progress over months to years to become oxygen-dependent, culminating in respiratory failure. An obstructive pattern of pulmonary function tests, typical symptoms, and no evidence of infection are regarded as diagnostic of BO after BMT.

While it is considered that chest radiography of BO is usually normal but sometimes shows hyperinflation,[5] our case

TABLE 1. Laboratory Data at the Operation

WBC	5900/μL
RBC	314 10^4/μL
HB	10.8 g/dL
PLT	6.1 10^4/μL
CRP	0.05 mg/dL
ALB	3.5 g/dL
GOT	22 IU/dL
GPT	17 IU/dL
LDH	206 IU/dL
PaO2	99.3 torr
PaCO2	34.7 torr
PH	7.387
BE	−3.8
HCO3−	20.4

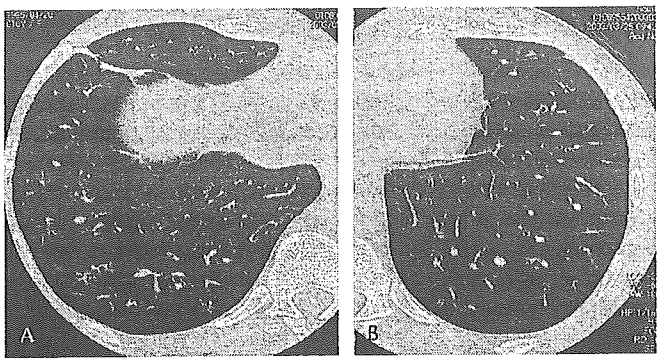

FIGURE 5. HRCT scan (1 mm collimation with high-resolution algorithm after the therapy demonstrates improved dilated thick-walled bronchioli and the centrilobular linear and branching opacities.

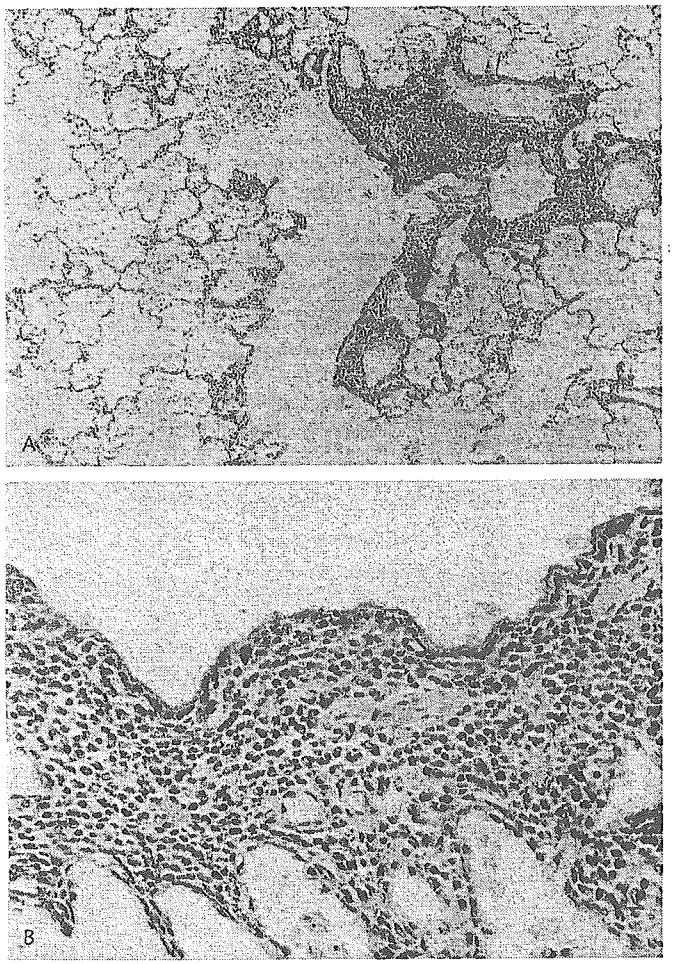

FIGURE 4. A, Photomicrography (hematoxylin and cosin, original magnification (×4) demonstrates a thickened wall of the distal bronchioli. B, Infiltrations of lymphocytes and plasma cells are seen in submucosal and intraepithelial lesion of the thickened bronchial wall (hematoxylin and cosin, original magnification (×40). Foamy macrophages were also involved in the alveoli near thickened bronchioli.

showed mild hyperinflation. HRCT is used for differential diagnosis of pulmonary complications after BMT.[6,7] Typical HRCT findings of BO after BMT are bronchial dilatation, a mosaic pattern of attenuation, and air trapping on expiratory scans.[5] Sargent et al reported that bronchial dilatation was more common in subsegmental than in segmental bronchi.[6] Ooi et al reported that normal, nonspecific bronchial dilatation and consolidation were typical findings of BO after BMT on HRCT,[11] although these findings were not specific. Histologically, there is predominantly constrictive bronchiolitis with a prebronchiolar inflammatory infiltrate of neutrophils and lymphocytes, which accounts for the air trapping seen on the expiratory CT scan.[5] In our case, HRCT suggested that the typical pathologic findings of BO and BOOP after BMT were not present.

We considered the final pathologic diagnosis of this case to be bronchiolitis without organizing changes resembling lymphocytic bronchiolitis. The pathologic changes mainly occurred in the periphery and there were more infiltrated macrophages, compared with BO after BMT. Furthermore, the presence of foamy macrophages and more peripheral bronchiolitis was similar to the pathology of DPB, but centrilobular peribronchiolar acute and chronic inflammatory cells and intraluminal inflammatory exudates, which are typical findings of DPB, were not present. The International Society of Heart-Lung Transplantation has revised a working formulation for the classification and grading of pulmonary rejection,[12,13] in which lymphocytic bronchiolitis is listed as airway inflammation. It is manifested by a patchy or diffuse submucosal infiltrate of lymphocytes and plasma cells.[14] Also, it has been reported that it may accompany or succeed the perivascular infiltrates of early rejection or be seen alone, and often responds to steroid treatment.[15] Based on the literature and the pathology, we decided that our patient had a new type of late pulmonary complication and the pathology was a variation of lymphocytic bronchiolitis. Thus, the corticosteroid treatment improved her clinical symptoms and radiographic appearances. Consequently, the HRCT findings were different from those of typical BO, and did not demonstrate an obstructive pattern.

GVHD occurs infrequently in patients after UCBT because the proliferative and cytotoxic responses of cord blood lymphocytes are blunted compared with those of adult peripheral lymphocytes.[3] However, our case had grade 2 chronic GVHD including lung complication. A case of fatal obstructive lung disease with severe GVHD after UCBT has been reported,[16] in which it was proposed that more investigation would be needed to analyze the degree of HLA disparities that is tolerable in UCBT. In our case, one antigen was mismatched since the pulmonary complications were progressive and the patient's condition was often not tolerable; open lung biopsy in bone marrow transplant patients is usually not attempted and it has been reported that it may not improve patient outcome, while histopathologic analysis is easy and accurate in determining the cause of pulmonary infiltrates.[17] We obtained open lung biopsy specimens, analyzed the histopathologic manifestations, and compared them to HRCT findings. While several types of HRCT findings on pulmonary complications after BMT have been reported, we think this is the first report of HRCT findings on this new type of pulmonary complication after UCBT. As it is speculated that the transplantation of umbilical cord blood may be a cause of developing a pulmonary complication such as ours, more investigations will be needed.

In conclusion, we experienced a new type of late pulmonary complication after UCBT and compared the HRCT findings and the pathology. This new type of late lung complication may occur more often with the increased frequency of UCBT.

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Risk of Pleural Recurrence After Needle Biopsy in Patients With Resected Early Stage Lung Cancer

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Background. Concerning the complications resulting from percutaneous needle biopsy (PNB), although cases of tumor seeding into the needle track have occasionally been reported, there were only two cases of pleural recurrences to date. The aim of this study was to elucidate the real risk of pleural recurrence after needle biopsy in patients with resected early stage lung cancer.

Methods. Between 1986 and 2000, 335 patients with stage I nonsmall cell lung cancer underwent complete resection of the lung tumor. We retrospectively reviewed their medical records and investigated the relationship between the diagnostic methods used and the cancer recurrence patterns.

Results. Preoperative diagnoses were obtained for 290 patients; 220 were diagnosed by bronchoscopy and 66 by PNB. Among the patients without a preoperative diagnosis, 27 were diagnosed by intraoperative needle biopsy and 14 by wedge resection of the lung. Tumors diagnosed by needle biopsy including PNB and intraoperative needle biopsy were smaller and showed less vessel invasion than those diagnosed by other methods ($p < 0.01$). After surgical resection, 9 patients had pleural recurrence and 1 patient, needle track implantation. Seven of these 10 patients were diagnosed by needle biopsy using 18G cutting type needle. Pleural recurrence or needle track implantation was observed for 8.6% of the patients who underwent a needle biopsy, whereas it was 0.9% for patients who were examined using other diagnostic modalities ($p = 0.0009$).

Conclusions. Needle biopsy especially using a cutting-type biopsy needle can cause a pleural recurrence in addition to needle track implantation.

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Percutaneous needle biopsies (PNB) are widely used for the histologic diagnosis of a peripheral indeterminate pulmonary nodule. The overall sensitivity and specificity for diagnosing peripheral lung cancers were 90% and 97% respectively by meta-analysis [1], and even for tumors less than 2 cm in diameter the sensitivity was also as high as 91%.

Although fine-needle aspiration (FNA) is widely used method for performing PNB around the world, automated or semiautomated cutting needles have been tested to increase the diagnostic yield [2–8]. We also previously reported on the usefulness of computed tomographic fluoroscopy-guided transthoracic needle biopsy using an 18G automatic biopsy gun for diagnosing pulmonary lesions, particularly benign lesions [9].

The most frequent complication of PNB is pneumothorax, which occurs for 25% to 30% of patients [10]. For fatal complications of PNB, air embolism and tumor seeding have been previously documented to occur. Cases of needle track implantation accounted for almost all of the cases of tumor seeding, and have been documented to occur at a rate of 0% to 3% [11–13]. Although pleural recurrence due to tumor seeding is a possible adverse event that may occur after PNB [14], only two such cases have been reported [15]. Pleural recurrence after PNB tends to be ascribed to the advanced disease itself a priori, but not to PNB, because malignant pleural effusion or tumor dissemination in the pleural cavity can be seen after usual lung surgery without performing a needle biopsy, especially for patients with locally advanced nonsmall cell lung cancer (NSCLC). Thus, in the present study we investigated the risk of pleural recurrence after needle biopsy for patients with pathologic stage I NSCLC, who were thought unlikely to experience recurrence in the pleural cavity after resection.

Material and Methods

Patients

Between October 1986 and December 2000, 687 patients with NSCLC underwent surgical resection of the lung at our hospital. Among them, 335 had pathologic stage I disease, and they constituted the study population. Two hundred patients were men, and the median age was 67 years (range, 35 to 85). The majority of the patients underwent a lobectomy with systematic nodal dissection (n = 256, 76%). Histologic types were adenocarcinoma (n = 222), squamous cell carcinoma (n = 89), and others (n = 24), including large cell carcinoma, large cell neuroendocrine carcinoma, adenosquamous carcinoma, carcinoid, and carcinosarcoma. Primary tumors were classified as T1 in 210 patients and T2 in 125 patients.

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Treatment Policy

Our routine diagnostic strategies for patients with an indeterminate pulmonary nodule were as follows. First, we obtained a histologic or cytologic diagnosis using fiberoptic bronchoscopy. If this failed or was difficult, the patients were then scheduled for diagnosis using PNB under computed tomography (CT) guidance. Almost all biopsies have been performed using an 18G, spring-loaded, automatic biopsy gun with a modified Tru-Cut type needle (Monopty; Bard Radiology, Convington, Georgia) since 1994 (n = 37), while the Tokyo Medical College needle (n = 12) and the Sure-Cut needle (n = 8) were frequently used before that time. If the state of the nodule remained undetermined, a diagnostic thoracotomy or thoracoscopy was subsequently performed. Some patients underwent intraoperative needle biopsy (INB) whereas the others underwent wedge resection of the lung. If a tumor was diagnosed as NSCLC by intraoperative pathology, the patient subsequently underwent complete resection of the tumor with curative intent. After surgery, the patients were scheduled for checkups, chest radiography, and measurement of serum tumor markers every 1 to 3 months for the first 2 years and every 6 months thereafter. When recurrence was discovered, intrathoracic and extrathoracic lesions were always surveyed.

Assessment of Recurrence and Clinicopathologic Features

We reviewed the medical records of all patients to confirm that recurrence had developed. Pleural recurrence was defined as pleural nodule or malignant effusion or both in the hemithorax of the operated side at the first relapse. Malignant effusion was diagnosed cytologically and pleural dissemination was diagnosed if multiple enhanced pleural nodules were observed on chest CT. Patients with any other site of recurrence in combination with pleural recurrence at the first relapse were included among the pleural recurrence cases, because we can not determine which recurrence preceded and caused the other recurrence. To elucidate the difference of tumor characteristics in each diagnostic group, we reviewed the CT images if available (n = 298) and classified these tumors into two categories according to their locations. When the center of a tumor shadow fell within the inner half of the lung, the tumor was classified as being central; and when the center of a tumor shadow fell within the outer half of the lung, the tumor was classified as being peripheral. We checked whether the tumor shadow was touching the pleura or not on the CT image and then examined the pathologic tumor characteristics such as tumor size, lymphatic invasion, and vascular invasion in the tumor in relation to the diagnostic method used. Pathologic stages were classified according to the criteria set forth by the International System for Staging Lung Cancer [16], and histologic typing was determined according to the World Health Organization classification [17].

Statistical Analysis

Correlations between the diagnostic methods used and the tumor characteristics were examined using the χ^2 test and Fisher's exact test. The unpaired t test was used to examine the relationship between the diagnostic methods used and the log-transformed tumor sizes because of their skewed distribution. All statistical analyses were carried out using STATA software [18].

Results

Among the 335 patients, 290 were diagnosed as having NSCLC preoperatively. Among them, the definitive diagnostic methods used were bronchoscopy for 220, PNB for 66, and sputum cytology for 4. Among the 45 patients with a pulmonary nodule without definitive preoperative diagnosis, INB was performed on 27, wedge resection of the lung on 14, and lung resection with curative intent without definitive diagnosis on 4.

Tumor Characteristics and Diagnostic Methods

The relationships between the methods used to obtain pathologic diagnoses and tumor characteristics are shown in Table 1. Tumors diagnosed by PNB showed less lymphatic invasion and were smaller than those diagnosed by bronchoscopy, and tumors diagnosed by INB were smaller and less invasive than those diagnosed by PNB. When we divided the tumors into two groups according to whether needle biopsy was conducted or not, we found that the needle biopsy group was associated with peripheral location, a smaller tumor size, and a lower occurrence of lymphatic and vascular invasion. Although tumors in the patients of the needle biopsy group were located in more peripheral areas, the numbers of tumors touching the pleura were almost identical. Furthermore, the incidence of pleural invasion of the tumors, which was thought to be associated with pleural recurrence, was lower for the needle biopsy group.

Recurrence

Two hundred and ninety-seven patients (88.7%) were followed up until February 29, 2004. Among the 38 patients not followed-up, 34 completed their follow-up after the 5-year anniversary of surgery. The median length of the follow-up period was 80 months, and the relationships between the methods used for histologic diagnosis and the recurrence patterns are shown in Table 2. Seventy-three patients were diagnosed as having recurrence, where the recurrence pattern was distant for 53 and local for 23. Among them, 3 had distant and local recurrence. Nine patients died of unknown causes; and as 1 patient was diagnosed with distant recurrence at an other hospital, we did not know whether pleural recurrence had developed. For the patients with local recurrence, 10 had pleural recurrence or needle track implantation. The percentage of cases for which distant recurrence had developed was similar between patients diagnosed by bronchoscopy and those diagnosed by PNB (18.5% versus

Table 1. Relationships Between Diagnostic Methods Used and Clinicopathologic Tumor Characteristics

	Diagnostic Methods								
	Preoperative			Intraoperative or Postoperative					
	Bronchoscopic	PNB	Sputum	INB	Wedge	Post	Needle	Nonneedle	*p* Value[a]
Total	220	66	4	27	14	4	93	242	
Clinical									
Sex									
Male	137 (62)	37 (56)	3 (75)	12 (44)	8 (57)	3 (75)	49 (53)	151 (62)	0.105
Female	83 (38)	29 (44)	1 (25)	15 (56)	6 (43)	1 (25)	44 (47)	91 (38)	
Age (years)									
<67	90 (41)	39 (59)	1 (25)	19 (70)	10 (71)	2 (50)	58 (62)	103 (43)	0.001
≥67	130 (59)	27 (41)	3 (75)	8 (30)	4 (29)	2 (50)	35 (38)	139 (57)	
Location									
Peripheral	142 (74)	56 (95)	1 (25)	20 (77)	12 (92)	2 (50)	76 (89)	159 (75)	0.005
Central	50 (26)	3 (5)	3 (75)	6 (23)	1 (8)	2 (50)	9 (11)	54 (25)	
Contact with the pleura									
Yes	91 (47)	32 (54)	2 (50)	8 (30)	6 (46)	1 (25)	40 (47)	100 (47)	0.986
No	101 (53)	27 (46)	2 (50)	18 (70)	7 (54)	3 (75)	45 (53)	113 (53)	
Pathologic									
Histology									
Adenocarcinoma	126 (57)	54 (82)	1 (25)	23 (85)	14 (100)	4 (100)	77 (83)	145 (60)	<0.001
Other	94 (43)	12 (18)	3 (75)	4 (15)	0 (0)	0 (0)	16 (17)	97 (40)	
T factor									
T1	121 (55)	45 (68)	0 (0)	26 (96)	14 (100)	4 (100)	71 (76)	139 (57)	0.001
T2	99 (45)	21 (32)	4 (100)	1 (4)	0 (0)	0 (0)	22 (24)	103 (43)	
Lymphatic invasion									
No	179 (81)	62 (94)	4 (100)	27 (100)	14 (100)	4 (100)	89 (96)	201 (83)	0.002
Yes	41 (19)	4 (6)	0 (0)	0 (0)	0 (0)	0 (0)	4 (4)	41 (17)	
Vascular invasion									
No	138 (63)	49 (74)	1 (25)	26 (96)	14 (100)	3 (75)	75 (81)	156 (64)	0.004
Yes	82 (37)	17 (26)	3 (75)	1 (4)	0 (0)	1 (25)	18 (19)	86 (36)	
Pleural invasion									
No	161 (73)	50 (76)	2 (50)	26 (96)	14 (100)	3 (75)	76 (82)	180 (74)	0.156
Yes	59 (27)	16 (24)	2 (50)	1 (4)	0 (0)	1 (25)	17 (18)	62 (26)	
Tumor size (cm)[b]	2.90	2.38	3.95	1.70	1.20	1.91	2.15	2.75	<0.001

[a] The *p* value for the χ^2 test for the association between needle biopsy and clinicopathologic characteristics in Table 1; [b] Geometric mean.

The numbers in parentheses indicate percentages.

INB = intraoperative needle biopsy; PNB = percutaneous needle biopsy.

15.2%). However, the rate of pleural recurrence for the cases diagnosed by PNB was significantly higher than for the cases diagnosed by bronchoscopy (9.1% versus 1.0%, $p < 0.0028$). The rate of distant recurrence for the cases diagnosed by INB was small at 3.7%, but the proportion with pleural recurrence among the cases diagnosed by INB was as high as that for the cases diagnosed by PNB, at 7.4%. Combining the cases diagnosed by PNB with those diagnosed by INB into the needle biopsy group, the percentage of those affected by pleural recurrence for the needle biopsy group was significantly higher than that for the cases diagnosed using other diagnostic modalities (8.6% versus 0.9%, $p = 0.0009$).

The details of these 10 cases are shown in Table 3. All 10 tumors were adenocarcinoma; and the diagnostic methods used were PNB for 6, INB for 2, and fiberoptic bronchoscopy for 2 patients. Two tumors diagnosed by bronchoscopy showed pleural and vessel invasion that may have been related to pleural recurrence. On the other hand, all 5 pleural recurrence cases showing neither pleural invasion nor vessel invasion in the primary tumor were diagnosed by PNB or INB. The average size of the tumors was 2.7 cm with a range from 1.5 cm to 4.8 cm, and the depth from the visceral pleura to the tumor surface on the needle track during needle biopsy ranged from 0 cm to 2.5 cm. Only 1 patient underwent needle biopsy directly through the

Table 2. Number of Cases According to Recurrence Pattern and Diagnostic Methods Used

	Diagnostic Methods							
	Preoperative			Intraoperative or Postoperative				
	Bronchoscopy	PNB	Sputum	INB	Wedge	Post	Needle	Nonneedle
Number of patients	220[a]	66	4	27	14	4	93	242[a]
Recurrence	48 (22.7)	17 (25.8)	3 (75)	3 (11.1)	2 (14.3)	0 (0)	20 (21.5)	53 (22.7)
Distant	39 (18.5)	10 (15.2)	2 (50)	1 (3.7)	1 (7.1)	0 (0)	11 (11.8)	42 (18.0)
Local	11 (5.2)	8 (12.1)	1 (25)	2 (7.4)	1 (7.1)	0 (0)	10 (10.8)	13 (5.6)
Both distant and local	2	1	0	0	0	0	1	2
Pleural recurrence	2 (1.0)[b]	6 (9.1)	0 (0)	2 (7.4)	0 (0)	0 (0)	8 (8.6)	2 (0.9)[b]
Pleural recurrence alone	2 (1.0)[b]	4 (6.1)	0 (0)	1 (3.7)	0 (0)	0 (0)	5 (5.4)	2 (0.9)[b]

[a] Bronchoscopy group includes 9 uninformative cases for recurrence. Recurrence percentages were calculated excluding the uninformative cases. [b] One patient was uninformative for pleural recurrence.

The numbers in parentheses indicate percentages.

INB = intraoperative needle biopsy; PNB = percutaneous needle biopsy.

pleura attached to the tumor, and for only 2 (cases 1 and 4) the distances were less than 1 cm. In regard to surgical procedures carried out in the 10 patients, lobectomy was performed in 7 patients and segmentectomy in 3 patients (cases 3, 7, and 10). Video-assisted thoracic surgery approach was applied in only 1 INB case; however, subsequent resection was carried out under an open thoracotomy. Concerning the pneumothorax and hemothorax after PNB, 2 cases of pneumothoraces were observed among the 6 patients who underwent PNB. Their relapses occurred 12 to 69 months after surgery, and only 2 patients (cases 4 and 8) with a short follow-up period remained alive with the recurrence.

Comment

A number of needle track implantation cases have been reported, and the incidences were reported at 0% to 3% [12, 13, 19–22]. This rate was considered as negligible by some researchers [12, 23, 24] and as important by the others [15, 25, 26]. On the other hand, pleural recurrence after PNB has not been recognized as a real risk of PNB, although it is theoretically possible adverse event. Only two cases of pleural recurrence after PNB were previously reported [15]. Is the real risk of pleural recurrence due to PNB extremely low? We thought that many cases of pleural recurrence due to PNB may have not been reported because of the difficulty in proving its cause. We

Table 3. Clinicopathologic Characteristics of 9 Cases With Pleural Recurrence and Needle Track Implantation

Case No.	Age (years)/Sex	Diagnostic Methods	Pathologic Findings			Concomitant Recurrence	Time to Recurrence (mo)	Outcome
			Histology	Size (cm)	P/Ly/V			
1	67/M	PNB	P/D Ad	2.2	0/−/−	No	20	DOD
2	68/F	INB	M/D Ad	2.8	0/−/−	Lymphadenopathy	13	DOD
3	72/F	INB	W/D Ad	1.5	0/−/−	No	12	DOD
4	58/F	PNB	M/D Ad	1.9	0/−/−	Pulmonary metastasis	36	AWD
5	50/M	PNB	P/D Ad	4.8	1/−/+	Lymphadenopathy	12	DOD
6	81/F	PNB	M/D Ad	2.5	2/−/−	Lymphangitis	28	DOD
7*	80/F	PNB	W/D Ad	2.6	2/−/−	No	24	DOD
8	76/M	PNB	W/D Ad	1.7	0/−/−	No	69	AWD
9	67/F	Br	P/D Ad	3.2	1/−/+	No	18	DOD
10	74/M	Br	M/D Ad	2.4	1/−/+	No	19	DOD

[a]Needle track implantation case.

Pleural invasion was judged as being P0 when tumor cells did not invade across the visceral elastic layer, P1 when tumor cells invaded across the visceral elastic layer, and P2 when tumor cells were exposed on the pleural surface.

AWD = alive with disease; Br = bronchoscopy; DOD = dead of disease; F = female; INB = intraoperative needle biopsy; Ly = lymphatic invasion; M = male; M/D Ad = moderately differentiated adenocarcinoma; P = visceral pleural invasion; P/D Ad = poorly differentiated adenocarcinoma; PNB = percutaneous needle biopsy; V = vascular invasion; W/D Ad = well-differentiated adenocarcinoma.

therefore conducted this investigation to elucidate the real risk of pleural recurrence after PNB. We hypothesized that pleural recurrence among patients with resected p-stage I NSCLC, especially with no pleural invasion, lymphatic invasion, and vascular invasion, was less likely to occur after surgery. Pleural recurrence, however, was noted in 9 patients. In addition, 1 case of needle track implantation was found. Among them, 5 cases without pleural and vessel invasion were diagnosed by needle biopsy, and for all an 18G cutting-type needle was used. These results suggested that PNB using this type of needle can cause a plural recurrence in addition to needle track implantation.

Another possible explanation for this high rate of pleural recurrence among the patients in the needle group is the difference in tumor biology between the two groups. Some investigators may believe that tumors diagnosed by PNB are in a peripheral location, and that this may be related to the high rate of pleural recurrence. From our results, we actually found that a peripheral location was more frequently observed for patients in the needle biopsy group. However, the numbers of tumors touching the pleura seen by chest CT were similar for both groups, and pathologic pleural invasion was less frequently observed for those in the needle group. We thought that the smaller size of the tumors for the needle biopsy group contributed to these results, which suggested that the differences in the tumor characteristics did not influence the results. However, we can not exclude the possibility that other tumor characteristics that we did not investigate in this study may have influenced the differences we observed.

The type of the needle we used could influence our high incidence of pleural recurrence. Large-bore cutting needles were replaced by FNA to reduce complications. During the 1990s, since the emergence of the automated and semiautomated cutting needle with an 18G to 20G bore, the cutting needle was used again because of its easy handling and its greater harvest of tissue [3–8]. Some studies compared the accuracy of cutting needle biopsy with FNA and concluded that cutting needle biopsy greatly increases the diagnostic accuracy for cases of benign pulmonary disease [4–6, 8]. On the other hand, for malignant lesions, FNA has the same high diagnostic accuracy as a cutting needle when on-site cytopathology is available [8, 27–29]. In our institute, the automated cutting type biopsy needle was conducted from 1994, and we reported its usefulness for benign lesions [9]. However, in the results from our current study, we encountered one case of needle track implantation among the 66 needle biopsy cases. Although, the incidence of needle track implantation at 1.5% was within the range of the reported incidence, it was on the high side. That the highest incidence of needle track implantation was reported by Harrison and coworkers [21], who used cutting type biopsy needle, suggested that cutting type needle usage could contribute to the tumor seeding. Conversely, more than 10 cases of needle track implantation after FNA have been reported [19, 20, 24, 30–34] since the first reported case by Sinner and Zajicek [13]. Ayar and colleagues [35] conducted a questionnaire study to elucidate the predictive factor for needle track implantation. They collected data on more than 60,000 needle biopsy cases. Among the 8 needle track implantation cases discovered in this study, 5 needle track implantations occurred after the use of 19G to 22G needles, and they concluded that they could not find any predictive factor including needle bore size. The thoroughness of our follow-up could have been related to our high incidence of tumor seeding. Our early stage of this study population has also affected the results. Needle track implantation in patients with early stage lung cancer may be more noticeable when compared with those in patients with more advanced disease because other recurrences may precede and obscure the implanted lesions. The occurrence of pneumothorax or hemithorax after PNB might be associated with the development of pleural recurrence. However, the incidence of hemothorax and pneumothorax among the pleural recurrence cases was 0% and 33 % (2 of 6 patients), and these incidences were not higher than the incidences that we previously reported (0% and 42%) [9].

Only one similar investigation that dealt with the risk of pleural recurrence was reported by Sawabata and colleagues [36]. This group studied 239 patients with completely resected NSCLC of less than 3 cm in maximum diameter and reported that no pleural carcinomatosis occurred for 45 patients who underwent PNB by FNA and wedge resection of the lung. The difference between their study and ours was that their study population included only 22 cases diagnosed by needle biopsy and 71 (30%) with stage II or more advanced disease for which other forms of recurrence could have obscured pleural recurrence.

To avoid the tumor seeding, some researchers have used a coaxial method for which aspiration or the cutting needle passes through an outer needle that stick into the normal lung [5, 6, 28, 37]. However, the effectiveness of this method has not been demonstrated.

The retrospective approach of this study is a weak point. Therefore, we can not conclude from this study that needle biopsy should be avoided. However, the results call doctor's attention to the potential risks faced by needle biopsy and suggest the need for further investigations focusing on pleural recurrence after needle biopsy. To elucidate the real risk of needle biopsy concerning the tumor seeding according to the type of needle or needle size, pleural recurrence and needle track implantation have to be investigated prospectively for patients with early stage lung cancer in multi-institutional setting. Randomized control trial is an ideal method, if possible. When the real risks of pleural recurrence and needle track implantation are discovered, this information will be indispensable for patients who would undergo this needle biopsy.

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A Phase I/II Study Comparing Regimen Schedules of Gemcitabine and Docetaxel in Japanese Patients with Stage IIIB/IV Non-small Cell Lung Cancer

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Objective: Gemcitabine and docetaxel are non-platinum agents with activity in non-small cell lung cancer (NSCLC). This study was conducted to determine and evaluate the recommended regimen of gemcitabine–docetaxel and evaluated its efficacy and safety in chemonaive Japanese NSCLC patients.

Methods: In phase I, patients with stage IIIB/IV NSCLC were randomized and received either gemcitabine on days 1 and 8 plus docetaxel on day 1 or gemcitabine on days 1 and 8 plus docetaxel on day 8. The recommended regimen was the dose level preceding the maximum tolerated dose; once determined, patients were enrolled in phase II. Efficacy and toxicity were evaluated in all patients.

Results: Twenty-five patients were enrolled in phase I and six patients were given the recommended regimen; gemcitabine 1000 mg/m^2 on days 1 and 8 plus docetaxel 50 mg/m^2 on day 8. An additional 34 patients were enrolled into phase II and administered with the recommended regimen. The response rate was 32.2% [95% confidence interval (CI) 20.6–45.6%] overall and 30.0% (95% CI 16.6–46.5%) in patients with the recommended regimen (40 patients). Although grade 3 interstitial pneumonia was observed in two patients (5.0%) who received the recommended regimen, both recovered shortly after steroid treatment. No unexpected events were observed throughout this study.

Conclusions: Gemcitabine 1000 mg/m^2 on days 1 and 8 plus docetaxel 50 mg/m^2 on day 8 has comparable efficacy and more tolerable toxicities than previously reported platinum-based regimens. These results should be verified by a phase III study.

Key words: docetaxel – gemcitabine – non-small cell lung cancer

INTRODUCTION

Non-small cell lung cancer (NSCLC) is one of the most common malignant tumors, progresses in a short time period, has a bleak prognosis, and represents the leading cause of cancer death in the world. The number of patients with NSCLC is increasing, and most tumors are inoperable. Despite improvements in the detection and treatment of NSCLC, long-term survival is rare. Therefore, the development of new chemotherapy treatments is essential.

The use of single-agent and combination chemotherapy against NSCLC has been studied. Platinum-based regimens have shown high efficacy but at the cost of severe toxicities (1,2). Therefore, non-platinum agents such as gemcitabine, docetaxel, paclitaxel, irinotecan and vinorelbine have been developed and have proven their efficacies. Among the new agents, the combination of gemcitabine and docetaxel has emerged as one of the most promising, showing equivalent efficacy with, and less toxicity than, cisplatin-based chemotherapies (3).

Gemcitabine (2′-deoxy-2′,2′-difluorocytidine monohydrochloride) is a nucleoside antimetabolite against deoxycytidine. It is intracellularly metabolized to gemcitabine triphosphate.

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